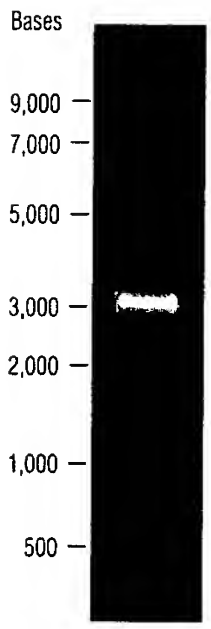
RNA Ladder

#N0362S 25 µg $55



1 µg of RNA Ladder was heated at 60°C for 3 minutes in 1X RNA Ladder Sample Buffer and visualized by ethidium bromide staining (1.0% TBE agarose gel).

Description: The RNA Ladder is a set of 7 RNA molecules produced by *in vitro* transcription of a mixture of 7 linear DNA templates. The ladder sizes are: 9000, 7000, 5000, 3000, 2000, 1000 and 500 bases. The 3000 base fragment is at double intensity to serve as a reference band. This ladder is suitable for use as an RNA size standard on denaturing or native agarose gels.

Reagents Supplied with Ladders:
2X RNA Ladder Sample Buffer (for use with native agarose gels)

2X RNA Ladder Sample Buffer:
2X TBE (pH 8.3), 13% ficoll (w/v), 0.01% bromophenol blue and 7 M urea.

Concentration: 500 µg/ml.

Storage Conditions: 20 mM KOAc (pH 4.5). Store at –70°C. For short term storage (< 1 week), ladder can be stored at –20°C.

Notes on Use:
To avoid ribonuclease contamination: wear gloves, use RNase-free water for gels and buffers, wash equipment with detergent and rinse thoroughly with RNase-free water.

It is best to use freshly poured gels that are as thin as possible (i.e, 2–10 mm). Excessively long run times or high voltage can cause degradation of the bands on the gel. We recommend 4–8 volts/cm and running the bromophenol blue approximately 5 cm into the gel for good resolution.

Adding ethidium bromide to agarose gels and running buffer at a final concentration of 0.5 µg/ml will effectively stain the bands during electrophoresis.

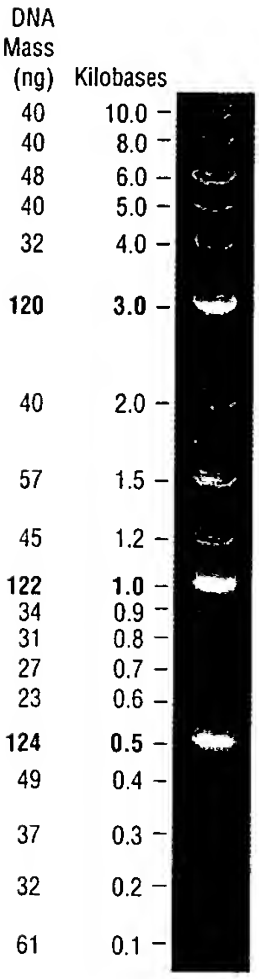
Denaturing vs. Native Agarose Gels:
It is common practice to electrophorese RNA on a fully denaturing agarose gel, such as one containing formaldehyde (1). However, in many cases it is possible to run RNA on a native agarose gel and obtain suitable results. In fact, it has been demonstrated that treatment of RNA samples in a denaturing buffer maintains the RNA molecules in a denatured state, during electrophoresis, for at least 3 hours (2,3). The use of native agarose gels eliminates problems associated with toxic chemicals and the difficulties encountered when staining and blotting formaldehyde gels.

References:
(1) Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, (2nd ed.), (pp. 7.43–7.45). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
(2) Liu, Y-C., Chou, Y-C. (1990) *Biotechniques* 9, 558.
(3) Sandra Cook and Christina Marchetti, unpublished observations.

2-Log DNA Ladder (0.1–10.0 kb)

New

#N3200S 100 µg $55
#N3200L 500 µg $220



1.0 µg of 2-Log DNA Ladder visualized by ethidium bromide staining on a 1.0% TBE agarose gel.

Description: A number of proprietary plasmids are digested to completion with appropriate restriction enzymes to yield 19 bands suitable for use as molecular weight standards for agarose gel electrophoresis. This digested DNA includes fragments ranging from 100 bp to 10 kb. The 0.5, 1.0 and 3.0 kb bands have increased intensity to serve as reference points.

Preparation: Double-stranded DNA is digested to completion with the appropriate restriction enzymes, phenol extracted and equilibrated to 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA.

Concentration: 1,000 µg/ml.

Storage Conditions: 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA. For long term storage, store at –20°C. 2-Log DNA Ladder is stable for at least 3 months at 4°C.

Note: All fragments have 4-base, 5´ overhangs that can be end labeled using T4 Polynucleotide Kinase (NEB #M0201) or filled-in using DNA Polymerase I, Klenow Fragment (NEB #M0210) (1). Use α-[^{32}P] dATP or α-[^{32}P] dTTP for the fill-in reaction.

Usage Recommendation: We recommend loading 1 µg of the 2-Log DNA Ladder diluted in sample buffer. This ladder was not designed for precise quantification of DNA mass but can be used for approximating the mass of DNA in comparably intense samples of similar size.

The approximate mass of DNA in each of the bands in our 2-Log DNA Ladder is as follows (assuming a 1 µg loading):

Fragment	Base Pairs	DNA Mass
1	10,002	40 ng
2	8,001	40 ng
3	6,001	48 ng
4	5,001	40 ng
5	4,001	32 ng
6	**3,001**	**120 ng**
7	2,017	40 ng
8	1,517	57 ng
9	1,200	45 ng
10	**1,000**	**122 ng**
11	900	34 ng
12	800	31 ng
13	700	27 ng
14	600	23 ng
15a	**517** >	**124 ng**
15b	**500**	
16	400	49 ng
17	300	37 ng
18	200	32 ng
19	100	61 ng

Reference:
(1) Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, (2nd ed.), (pp. 10.51–10.67). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.